bio-protocol Preprint

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Spheroid preparation for immunohistochemistry (IHC)

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An abbreviated version of this protocol was published in Translational Oncology in May 2021

3D heterospecies spheroids of pancreatic stroma and cancer cells demonstrate key phenotypes of pancreatic ductal adenocarcinoma

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Detailed protocol

Spheroids were collected following 5 days of cultivation, washed in phosphate buffered saline and fixed in 4% paraformaldehyde for 1 h at room temperature. Another PBS washing step is optional. Then spheroids were transferred to a corner of a biopsy cryomold (Tissue-Tek Cryomold #4565) with as little liquid as possible. One can remove extra liquid with a drawn out Pasteur pipette. HistoGel (ThermoFisher Scientific, HG-4000-012) was heated to 60°C to liquidify. About 100 µl HistoGel was used to embed spheroids at one corner of the cryomold, followed by addition of another 300 µl HistoGel to fill up the biopsy cryomold. Then, the biopsy cryomold was put on ice to solidify the gel. After that, the solid gel was gently transferred into a biopsy cassette and kept in 70% ethanol until further processing. Paraffin-embedded spheroids were sectioned at 4 □m before hematoxylin-eosin staining or immunohistochemistry.

How to cite:(Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Heuchel, R. (2022). Spheroid preparation for immunohistochemistry (IHC). Bio-protocol Preprint. bio-protocol.org/prep1930.
2. Liu, X., Gündel, B., Li, X., Liu, J., Wright, A., Löhr, M., Arvidsson, G. and Heuchel, R.(2021). 3D heterospecies spheroids of pancreatic stroma and cancer cells demonstrate key phenotypes of pancreatic ductal adenocarcinoma. Translational Oncology 14(7). DOI: 10.1016/j.tranon.2021.101107